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IgA Antiphospholipid Antibodies and Anti-Domain 1 of Beta 2 Glycoprotein 1 Antibodies are Associated with Livedo Reticularis and Heart Valve Disease in Antiphospholipid Syndrome

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. Antiphospholipid syndrome (APS) is an autoimmune disease associated with venous or arterial thrombosis and pregnancy loss, but also infrequently with non-criteria APS manifestations such as thrombocytopenia, livedo reticularis and heart valve disease. The occurrence of antiphospholipid antibodies is necessary to diagnose APS and includes the presence of lupus anticoagulant and anticardiolipin as well as anti- β 2-glycoprotein I antibodies, both in IgM and/or IgG isotype.

Objectives. The aim of this study was to evaluate the associations between antiphospholipid antibodies including IgA isotype and IgG anti-domain I of β 2-glycoprotein I (β -2GPI-D1) and non-criteria-related manifestations of APS.

Material and Methods. Thirty-three consecutive APS patients (26 women, 7 men, aged 44.1 ± 15 years), including 23 (69.7%) subjects with primary APS, were enrolled. Together with standard antiphospholipid antibodies, IgA anticardiolipin, IgA anti- β 2-glycoprotein I and IgG anti- β -2GPI-D1 antibodies in serum samples were evaluated by chemiluminescence using the QUANTA Flash® System.

Results. Livedo reticularis ($n = 8$, 24.2%) was associated with increased levels of IgG anti- β -2GPI-D1 ($p = 0.005$), IgA anticardiolipin ($p = 0.001$) and IgA anti- β 2-glycoprotein I ($p = 0.002$) antibodies. Heart valve disease ($n = 9$, 27.3%) was observed in patients with higher IgG anti- β -2GPI-D1 ($p = 0.01$). The associations of HVD with increased levels of IgA aCL and IgA anti- β -2GPI tended to be significant ($p = 0.07$). None of antiphospholipid antibodies showed association with thrombocytopenia ($n = 6$, 18.2%).

Conclusions. Our study suggests that increased IgA antiphospholipid antibodies and IgG anti- β -2GPI-D1 antibodies may be involved in the development of livedo reticularis and heart valve disease in APS patients (*Adv Clin Exp Med* 2014, 23, 5, 729–733).

Key words: antiphospholipid syndrome, beta 2 glycoprotein 1, domain 1, heart valve disease, livedo reticularis.

The antiphospholipid syndrome (APS) is an autoimmune disease associated with increased risk of venous thromboembolism or ischemic stroke and/or pregnancy loss [1, 2]. Other non-criteria APS manifestations include livedo reticularis (LR), thrombocytopenia and heart valve disease (HVD) [3]. These manifestations can be observed in 20–30% of the APS patients [4, 5].

Laboratory criteria of APS include the presence of lupus anticoagulant (LA) in plasma, the occurrence of anticardiolipin (aCL) antibodies of

IgG and/or IgM isotype in serum or plasma in medium or high titer as well as anti-beta-2-glycoprotein (β -2GPI) antibodies of IgG and/or IgM isotype in serum or plasma in titer over the 99th percentile, all detected on two or more occasions at least 12 weeks apart [6, 7].

IgA aCL and anti- β -2GPI antibodies have been reported in up to 70% of patients with systemic lupus erythematosus (SLE) and in those with primary APS [8]. The role of these antibodies in APS is, however, unclear. Lagos et al. [3] found that LR,

HVD, thrombocytopenia, and epilepsy are more common among subjects with increased IgA anti- β -2GPI antibodies. They also reported associations between increased IgA aCL antibodies and both LR and epilepsy [3]. A similar association was observed in SLE patients [9] especially in those with thrombocytopenia [10].

Another antiphospholipid antibody, IgG anti-domain I of β_2 -glycoprotein I (β -2GPI-D1), was reported in APS patients with no associations with any non-criteria APS manifestations [11–13].

We sought to investigate the associations of non-criteria APS manifestations with IgA isotype and IgG β -2GPI-D1 antibodies.

Material and Methods

Thirty-three APS patients, including 26 women, below 65 years of age were recruited between February 2012 to August 2013 in the Center for Coagulation Disorders in Krakow, Poland. The patients fulfilled the current criteria for APS [7]. Once the diagnosis of primary APS was established, coagulation treatment was recommended to all patients. The exclusion criteria were the following: acute infections, severe inflammatory disorders other than autoimmune disorders (e.g. nephrotic syndrome, inflammatory bowel disease), overt malignancy, use of oral contraceptives or hormone replacement therapy, atrial fibrillation, heart failure, diabetes mellitus, serum creatinine $> 120 \mu\text{mol/L}$. Deep vein thrombosis (DVT) was diagnosed with positive finding of color duplex sonography, whereas the diagnosis of pulmonary embolism (PE) was based on representative symptoms and positive results of high-resolution spiral computed tomography. Ischemic stroke was defined based on the WHO criteria. HVD was diagnosed based on echocardiographic evidence of thickening cardiac mitral and/or aortic valves. Patients with LR had permanent self-reported livedo on the trunks and extremities confirmed by the investigator (A.U.) at room temperature on 2 separate occasions. Thrombocytopenic patients had less than 100,000 platelets per 1 mm^3 if other known causes were excluded. All patients gave written informed consent.

All patients were screened for thrombophilia, including the factor V Leiden, prothrombin G20210A, antithrombin, protein C, free protein S. Inherited thrombophilia was defined as the presence of either of the two mutations or a deficiency of one of the three coagulation inhibitors. An evaluation of LA was performed using a clot-based assay according to the current criteria for LA detection [14]. A ratio more than 1.2 was considered

Table 1. Characteristics of APS patients

Variable	Total (n = 33)
Age – years	44.1 \pm 15.0
Male	7 (21.1)
SAPS	10 (30.3)
DVT	17 (51.5)
Recurrent pregnancy loss	8 (24.2)
PE	2 (6.1)
Stroke	8 (24.2)
CVST	6 (18.2)
Livedo reticularis	8 (24.2)
Heart valve disease	9 (27.3)
Thrombocytopenia	6 (18.2)
Inherited thrombophilia	10 (30.3)
ASA	3 (9.1)
LMWH	2 (6.1)
VKA	4 (12.1)
LA positive	10 (30.3)
IgG anti- β -2GPI (> 15.7 SGU)	11 (33.3)
IgM anti- β -2GPI (> 14 SMU)	16 (48.5)
IgG aCL (> 13.1 GPL)	16 (48.5)
IgM aCL (> 17.3 MPL)	15 (45.5)
IgA anti- β -2GPI (> 20 CU)	6 (18.2)
IgA aCL (> 20 CU)	6 (18.2)
anti- β -2GPI-D1 (> 19.9 CU)	10 (30.3)

Data are given as mean \pm standard deviation or number (percentage). ASA – acetylsalicylic acid; aCL – anticardiolipin; β -2GPI – beta-2-glycoprotein I; β -2GPI-D1 – domain I of beta-2-glycoprotein I; CU – chemiluminescent unit; CVST – cerebral venous sinus thrombosis; DVT – deep venous thrombosis; GPL – IgG phospholipid unit; LA – lupus anticoagulant; LMWH – low molecular weight heparin; MPL – IgM phospholipid unit; PE – pulmonary embolism; SAPS – secondary APS; SMU – standard IgM units; SGU – standard IgG units, VKA – vitamin K antagonist.

positive for LA. The levels of IgG and IgM aCL and anti- β -2GPI antibodies were determined by enzyme-linked immunosorbent immunoassays (QUANTA Lite™ ELISAs, INOVA Diagnostic, Inc, San Diego, CA). Reference ranges for IgG were up to 13.1 GPL and 15.7 SGU, respectively, and for IgM up to 17.3 MPL and 14 SMU, respectively. All positive cases were assessed after 12–16 weeks.

IgA antibodies isotypes of aCL, anti- β -2GPI

and IgG anti- β -2GPI-D1 in serum samples were determined by chemiluminescence on the BIO-FLASH® instrument (INOVA Diagnostic, Inc, San Diego, CA). Reference ranges for aCL, anti- β -2GPI were up to 20.0 CU and for anti- β -2GPI-D1 was up to 19.9 CU.

Depending on the distribution, as assessed by Shapiro-Wilk W test, quantitative values are expressed as median (interquartile range) or numbers with percentages. The relationship between qualitative parameters was assessed by the Fisher exact test. The Whitney-Mann U-test was used to evaluate the association between the studied antibodies levels and various APS manifestations. A p-value < 0.05 was considered statistically significant. Analysis was performed with STATISTICA Version 9.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

There were 23 (69.7%) patients with primary APS and 10 (30.3%) with secondary APS. At the enrolment visit, primary APS patients were on aspirin (n = 2), vitamin K antagonists (n = 3) or rivaroxaban (n = 1). Triple positivity was observed in 10 patients (30.3%), while there were 8 (24.2%) patients with double and 15 (45.5%) with single positivity. Characteristics of APS patients are presented in Table 1.

In patients with secondary APS, we observed increased levels of IgG anti- β -2GPI antibodies (p = 0.03) and more common positivity of LA (p = 0.09).

The presence of LR (n = 8, 24.2%) was related to a high proportion of thromboembolic manifestations of APS, including DVT, stroke and cerebral venous sinus thrombosis (4, 2, and 2 patients, respectively). As shown in Table 2, LR was associated with increased levels of IgA aCL (p = 0.001), IgA anti- β -2GPI (p = 0.002), IgG anti- β -2GPI-D1 (p = 0.005) and IgG anti- β -2GPI (p = 0.03) antibodies.

Occurrence of HVD (n = 9, 27.3%) was mainly related to DVT (6 patients) and was associated with elevated levels of IgG anti- β -2GPI-D1 (p = 0.01), IgG anti- β -2GPI (p = 0.002) and IgG aCL (p = 0.01) antibodies. The association of HVD with increased levels of IgA aCL and IgA anti- β -2GPI tended to be significant (p = 0.07).

Thrombocytopenia (n = 6, 18.2%) showed no associations with various types of antiphospholipid antibodies (Table 2).

No association between non-criteria APS manifestations and inherited thrombophilia, medications or thromboembolism were observed. In relation to both IgM isotypes no relationships were observed.

Table 2. The frequency of LA and levels of antibodies in patients with or without the non-criteria manifestations of APS

	LA	IgG anti- β -2GPI	IgM anti- β -2GPI	IgG aCL	IgM aCL	IgA anti- β -2GPI	IgA aCL	IgG anti- β -2GPI	IgA aCL	anti- β -2GPI-D1
With LR (n = 8)	4 (50)	16.05 (5.4-77.55)	7.95 (2.9-33.05)	29.3 (8.7-64.1)	14.3 (8.35-41.85)	46.35 (7.3-68.65)	46.9 (6.1-70.3)	78 (11.85-358.1)	46.9 (6.1-70.3)	78 (11.85-358.1)
Without LR (n = 25)	6 (24)	3.5 (1.8-5.3)	13.8 (2.6-44.1)	8.5 (3.9-26.2)	15.9 (9.9-34.4)	4 (4.0-4.0)	1.4 (1.4-1.9)	3.6 (3.6-4.9)	1.4 (1.4-1.9)	3.6 (3.6-4.9)
P-value	0.33	0.03	0.88	0.22	0.71	0.002	0.001	0.005	0.001	0.005
With HVD (n = 9)	6 (66.7)	12.6 (8.1-84.5)	5.9 (2.0-16.0)	72.3 (25.1-75.2)	22.6 (12.9-44.1)	9.3 (4.0-19.0)	5.9 (1.6-16.4)	41.4 (20.5-239.0)	5.9 (1.6-16.4)	41.4 (20.5-239.0)
Without HVD (n = 24)	4 (16.7)	2.9 (1.25-5.05)	15.1 (2.7-41.45)	7.35 (3.8-25.1)	15.2 (8.8-31.5)	4 (4.0-4.15)	1.4 (1.4-2.15)	3.6 (3.6-4.7)	1.4 (1.4-2.15)	3.6 (3.6-4.7)
P-value	0.06	0.002	0.6	0.01	0.38	0.07	0.07	0.01	0.07	0.01
With thrombocytopenia (n = 6)	3 (50)	7.1 (1.8-12.6)	7.45 (2.6-58.0)	48.45 (4.3-73.5)	29.8 (13.7-46.5)	5.65 (4.0-19.0)	3.85 (1.4-16.4)	48.8 (3.6-135.5)	3.85 (1.4-16.4)	48.8 (3.6-135.5)
Without thrombocytopenia (n = 27)	7 (25.9)	3.7 (2.1-13.5)	13.8 (2.3-30.4)	11.7 (3.9-33.3)	13.2 (7.7-22.6)	4 (4.0-10.6)	1.4 (1.4-9.8)	3.6 (3.6-20.5)	1.4 (1.4-9.8)	3.6 (3.6-20.5)
P-value	0.42	0.77	0.89	0.32	0.1	0.63	0.51	0.34	0.51	0.34
With SAPS (n = 10)	6 (60)	9.9 (5-84.5)	14.9 (7.4-58)	30.65 (4.3-75.2)	20.1 (13.7-36.6)	10.95 (4-41)	9.9 (1.4-56.5)	20.55 (3.6-135)	9.9 (1.4-56.5)	20.55 (3.6-135)
Without SAPS (n = 23)	4 (17.4)	3.4 (1.4-8.1)	3.5 (2-38.8)	8.5 (3.7-26.2)	12.9 (8.1-34.4)	4 (4-4.3)	1.4 (1.4-2.4)	3.6 (3.6-14.4)	1.4 (1.4-2.4)	3.6 (3.6-14.4)
P-value	0.09	0.03	0.18	0.15	0.3	0.11	0.09	0.14	0.09	0.14

Data are given as number (percentage), or medians (interquartile range) as appropriate. Statistical significance of the differences between patients with and without the symptoms of APS were assessed by Fisher's exact test or Mann-Whitney U-test. HVD – heart valve disease; LA – lupus anticoagulant; LR – livedo reticularis; SAPS – secondary APS; other see Table 1.

Discussion

A novel finding of this study is that increased IgG anti- β -2GPI-D1 antibodies were associated with the occurrence of LR and HVD in APS.

In contrast to the previous studies [3, 9, 10], we did not observe any relationships between increased IgA aCL and anti- β -2GPI antibodies and thrombocytopenia. In turn, the association of HVD with increased levels of IgA aCL and IgA anti- β -2GPI approaching only borderline significance. However, since there were 2 patients with HVD and 1 with thrombocytopenia with increased IgA aCL and anti- β -2GPI antibodies, we cannot exclude that in a larger patient group such association can be found.

LR related to poorly defined vascular abnormalities can be a strong marker of thrombosis in APS [15]. We observed specific associations between LR and increased levels of 3 types of antibodies, i.e. IgA aCL, IgA anti- β -2GPI and IgG anti- β -2GPI-D1 antibodies, which might indicate

that these antibodies unfavorably affect vascular reactivity.

Interestingly, the presence of IgG anti- β -2GPI-D1 antibodies was associated with HVD, which has been shown to have a link to thrombotic processes on valves with autoimmune valvulitis, being a risk factor for stroke [1]. It might be speculated that these antibodies displaying an uncertain pathogenic role may be implicated in the development of this particular manifestation of APS.

In conclusion, our study is the first to show that increased IgG anti- β -2GPI-D1 antibodies are associated with LR and HVD in APS patients. Comparing the three variables: levels of studied antibodies, rare symptoms and clinical manifestations measurement of both IgA and IgG anti- β -2GPI-D1 antibodies may be helpful in diagnosing patients with symptoms suggestive of APS, especially when standard IgG and IgM of antiphospholipid antibodies were negative.

The limitation of the study was the small sample size. Further confirmation of our findings in larger cohorts is needed.

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